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(54) Title: METHOD FOR TREATMENT OF INVASIVE CELLS

(57) Abstract

A method for treating metastatic tumor cells of a subject is disclosed. The method comprises administrating to the subject an antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein, or an antibody molecule capable of binding to a PAR protein. A method is also described for the treatment of disorders involving the implantation of a placenta in a female subject comprising administrating to the subject the antisense molecule. Also disclosed is the antisense molecule and a pharmaceutical composition comprising it.

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METHOD FOR TREATMENT OF INVASIVE CELLS

FIELD OF THE INVENTION

This invention relates to the therapeutic use of molecules associated with protease activated receptors.

BACKGROUND OF THE INVENTION

References referred to by bracketed numbers in the body of the specification are listed at the end of the specification before the claims.

The process by which epithelial cells become invasive is complex and has yet to be fully elucidated. One example of this process is observed in metastatic tumors. Another example of epithelial cells becoming invasive occurs during normal human embryonic development, in which the cytotrophoblasts (i.e. the fetal cells on the front line of the placenta) invade the uterus, as part of their normal differentiation program and successful implantation.

The physiologic invasiveness of cytotrophoblasts closely resembles that of malignant cells, sharing many common features. Tumor invasion and metastasis involve, among other alterations, proteolytic modification of basement membranes and extracellular matrices (ECMs). Cancer cells have to detach from their primary location, encounter basement membranes (i.e. during extravasation of blood or lymphatic vessels), and disseminate through the circulation to establish new cellular colonies at distant sites. Therefore, the process of cell invasion involves a well-orchestrated sequence of events including integrin activation, cell migration and proteolytic degradation of specific barrier components. This enzymatic cleavage is highly regulated, since extensive proteolysis could impede the invasive process by degrading essential

matrix components required for the transmission of survival and cell shape signals, through contacts with the basement membrane. Localized proteolysis directed to discrete regions of the cell surface may facilitate cellular invasion.

The thrombin-receptor (ThR) is a seven transmembrane domain G-coupled protein, belonging to the protease-activated receptor (PAR) family **[1]**. Recently, two other members of this family (PAR-2 and PAR-3) have been identified **[2-4]**, and a fourth member (PAR-4) has also been described **[19]**. Unlike most cellular growth factor receptors, the activation of these receptors does not require formation of the traditional ligand-receptor complex. Instead, the receptor serves as a substrate for proteolytic digestion, yielding an irreversible form of activated cell surface protein to convey further cell signaling.

Applicant's co-pending Israel Patent Application No. 114890, whose contents are incorporated herein by reference, discloses that a direct correlation exists between ThR level of expression in tumor cells and their degree of invasiveness. This finding was used to develop a diagnostic method for evaluating the metastatic tendency of tumor cells by following the expression of the ThR gene.

U.S. 5,352,664 to Carney, *et al*, describes thrombin-derived polypeptides which are capable of selectively stimulating or inhibiting thrombin receptor occupancy signals. Carney suggests that the inhibitory polypeptides may be used in preventing metastasis and angiogenesis. No supporting data is disclosed.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a method for treating metastatic tumors.

It is a further object of the present invention to provide a method for treating irregularities in physiological placental development.

The present invention is based on the surprising finding that interfering with the expression of PAR proteins of an invasive cell affects its degree of invasiveness. The interference may be realized at the DNA (gene) level, at the mRNA level, and/or at the protein (receptor) level. Interference at the DNA level may be achieved by use of gene therapy methods; interference at the mRNA level may be achieved by use of antisense molecules; and interference at the protein level may be achieved by use of specific antibodies.

The PAR protein may be any member of the PAR family such as, for example but not limited to, ThR, PAR-2, PAR-3 and PAR-4.

In a first aspect of the invention, the invasive cells are pathological cells such as metastatic tumor cells. Thus, in this aspect of the invention, there is provided a method for treating metastatic tumor cells of a subject comprising administrating to said subject an antisense molecule, said antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a PAR protein.

Also provided are antisense molecules and pharmaceutical compositions comprising them.

Further provided is a method for treating metastatic tumor cells of a subject comprising administrating to said subject an antibody molecule, said antibody molecule being capable of binding to a protease activated receptor (PAR) protein. The antibody molecule may be a polyclonal or monoclonal antibody, prepared by methods known *per se*.

In this aspect of the invention, the tumor cells will generally be of epithelial origin, which form solid carcinoma-type tumors. Examples of such epithelial tissues are breast, esophagus, kidney, prostate, ovary, melanoma and bladder tissue.

In a second aspect of the invention, the invasive cells are normal cells such as placental cells. As described above, ThR plays a role during cytotrophoblast invasion and implantation. The finding that ThR expression is associated with the invasiveness of placental tissue may be beneficial for

improved implantation of human embryo in the maternal uterus decidua. To date, the rate of spontaneous abortions is 8-12%, 50% of which are due to defects in proper implantation. It is even more striking in the I.V.F. procedure, where 40% of the overall cases result in failure. 90% of these failures are apparently due to implantation defects. Transfection of normal placenta with ThR and other PAR family genes may considerably improve implantation.

Thus, in this aspect of the invention, there is provided a method for the treatment of disorders involving the implantation of a placenta in a female subject comprising administrating to said subject an antisense molecule, said antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a PAR protein.

Also provided are antisense molecules and pharmaceutical compositions comprising them.

The synthesis of antisense molecules to known mRNA sequences is well known to the skilled artisan. In theory, based on Watson-Crick base pair formation, if an appropriate target can be identified, an antisense oligomer of more than 15 to 17 nucleotides in length would be expected to have a unique sequence relative to the entire human genome. A suitable oligomer should be able to interfere, in a sequence specific manner with the process of mRNA translation into protein **[9]**. The requirements for an antisense oligomer for therapeutic use are: (1) that it must be stable *in vivo; (2)* it must be able to enter the target cell; and (3) it must be able to interact with its cellular targets.

As oligomers possess little or no innate ability to diffuse across cell membranes, the cells must take them up through energy-dependent mechanisms. To resolve the problem of uptake, a large number of strategies have been employed in order to augment the rate of cellular internalization of nucleic acids and to increase the rate at which they pass through the endosomal membrane. These strategies include: (i) coupling oligomers to polycations such as polylysine **[10]**, polyethylamine **[11]** or others; (ii) use of transferin/polylysine-conjugated DNA in the presence of the capsid of a

replication-deficient adenovirus **[12]**; (iii) conjugation of oligonucleotides to fusogenic peptides **[13]** or to a peptide fragment of the homeodomain of the *Drosophila* antennapedia protein **[14]**; (iv) targeting of oligonucleotides to specific cell surface receptors, such as folate, asialoglycoprotein receptor and transferrin **[15]**, (v) conjugation to cholesterol **[16]**; and, most successfully (vi) complexation of oligonucleotides with cationic lipids **[17]** and GS 288 etofectin **[18]**.

Preferred antisense sequences are those designed to comprise sequences which hybridize to uniquely conserved regions in the PAR family of proteins. Conserved regions may be identified by comparing the nucleotide sequences of different members of the PAR family. For example, certain regions within the ThR sequence have 27% sequence similarity to PAR-3 and 28% similarity to PAR-2. Examples of conserved unique regions are:

1) The protease activated domains and hirudin binding domain:

	Nucleotides
hPAR-1(ThR)	37-61..... TLDPRSFLLRNPNDKYEPFWEDEEK
hPAR-2	32-56.....SSKGRSLIGKVDGTSHVTGKGVTVE
hPAR-3	34-57.....TLPIKTFRGAPPN SFEEFPFSALE
hPAR-4	28-52.....LPAPRGYPGQVCANDSDTHELPDSS

2) Second extracellular loop: located between transmembrane domains 4 & 5 and corresponding to residues: **ITTCHDV** which are conserved in PAR 1-3, while in PAR-4 only the three amino acids **CHD** are conserved.

3) The entire promoter region of the PAR family (i.e. 5′ cloned regions downstream to the ATG of PAR-1 and PAR-3). This region is likely to contain important regulatory sequences.

DETAILED DESCRIPTION OF THE DRAWINGS:

In order to understand the invention and to see how it may be carried out in practice, a preferred embodiment will now be described, by way of non-limiting example only, with reference to the accompanying drawings, in which:

Fig. 1 shows the DNA and amino acid sequence of human ThR **[1]**;

Fig. 2 shows the DNA sequence of an antisense cDNA of ThR;

Fig. 3 shows the location of the ThR antisense in the pcDNA III vector;

Fig. 4 illustrates ThR expression in human breast carcinoma cell lines. Total RNA isolated from human breast carcinoma cell-lines was analyzed by Northern blotting. The cell lines used were: MDA-435 (A), MDA-231 (B) and MCF-7 (C), as well as Ha-ras-transfected breast carcinoma cell lines, MCF10AT3B (D), MCF10AT (E) and MCF10A (F). The blots were probed with ^{32}P-labeled 250 base pair DNA, corresponding to ThR (upper part), or with ^{32}P-labeled β-actin DNA (lower part).

Fig. 5 illustrates immunocytochemical analysis of cell-associated ThR. Human breast carcinoma cell lines (MCF-7, MDA-231, and MDA-435) were cultured in 8-well chamber slides and analyzed for the presence of ThR. Specific staining of the receptor was obtained following incubation with affinity purified polyclonal anti ThR antiserum followed by biotin conjugated goat-anti-rabbit IgG antibodies and detected by extravidin incubation. Photographs of representative areas of MCF-7 (*a*), MDA-231 (*b*) and MDA-435 (*c*) cell monolayers are shown (x400).

Lower Panel. Western blot analysis of ThR. Western blot analysis of cell lysates (50μg/lane) of MCF-7 (A), MDA-231(B) and MDA-435 (C) cells. Specific protein band was detected following incubation with anti ThR antibodies and visualized by the ECL immunoblotting detection system according to the manufacturer's instructions.

Fig. 6 illustrates *in situ* hybridization of ThR mRNA in normal and cancerous breast tissue specimens. Hybridization with ThR riboprobes was performed on: Normal breast duct lobular units (*A&D*). Invasive duct carcinoma, (IDC) (antisense orientation, C; sense orientation, B). High grade DCIS of comedo type (antisense orientation, *E*; sense orientation, F). Low grade DCIS, solid type (G) and atypical intraductal hyperplasia (AIDH, H *&I*).

Detection of specifically hybridized mRNA to DIG-labeled probe was performed using anti-DIG-alkaline phosphatase conjugated antibodies (Boehringer Mannheim, Mannheim, Germany). These analyses represent at least 3 patients of each category.

Fig. 7 illustrates Matrigel invasion of breast carcinoma cell lines. The indicated cells (ZR-75, A; MCF-7, B; MDA-435, C; MDA-231, D; fibrocystic MCF10AT3B, E; fibrocystic MCF10A, F) were applied ($2x10^5$ cells/assay) to the upper compartment of Boyden chambers. Cell invasion through Matrigel coated filters was determined, as outlined in Materials and Methods, below.

Fig. 8 illustrates inhibition of MDA-435 Matrigel invasion by ThR antisense. MDA-435 cells were transiently transfected with pCDNAIII expression plasmid containing the antisense ThR of Fig. 2. The level of invasion was compared to untreated MDA-435 (A) and MCF-7 (B) cells. Control transfections of MDA-435 cells were performed in the presence of vector alone - (C) or DOTAP liposomes alone (Gibco -BRL) (D). Nearly confluent (60%) cells were treated with various concentrations of the plasmid: transfection with antisense ThR - 5 μg/plate (E), transfection with antisense ThR - 20 μg/plate (F). The invasion assay was performed as described under Materials and Methods, 72 h following transfection.

Lower panel. Western blot analysis of ThR antisense transfectants. MDA-435 cell lysates (50μg/lane) of ThR antisense transfectants (A) were applied on SDS-PAGE and the level of receptor protein was compared to cells transfected with vector alone (B) or untreated cells (C).

Fig. 9 shows the DNA sequence of PAR-2;

Fig. 10 shows the DNA sequence of PAR-3;

Fig. 11 shows the DNA sequence of PAR-4; and

Fig. 12 illustrates expression of ThR in first trimester human placenta. *In situ* hybridization analysis of ThR expression at 6-15 weeks of gestation. Placental tissue was obtained from elective termination of pregnancies by dilatation and curettage. Sections of 6 week placental tissue (A) and of 7, 8, 9

and 10 weeks of gestation (B-*E*, respectively), as visualized by ThR staining of cytotrophoblasts. No staining was observed at weeks 11 and 15 (F *&* *G*, respectively). Control hybridization (weeks 7 and 8) using sense orientation showed background staining (H *&* *I*, respectively).

DETAILED DESCRIPTION OF A PREFERRED EMBODIMENT

Materials and Methods

Cells: The human breast carcinoma cell lines, MCF-7 (adenocarcinoma), MDA-MB-231 (adenocarcinoma), MDA-MB-435 (ductal carcinoma) and ZR-75-1 (carcinoma), were kindly provided by Dr. Robert Stern (Department of Pathology, University of California, San Francisco). The invasive properties of these breast cell lines were determined following injection of the cells into the mammary pads of nude mice with or without Matrigel **[5]**. Cells were cultured in DMEM (1g glucose/liter) containing 10% bovine calf serum. MCF10A (nearly-normal immortalized epithelial cells), MCF10AT cells (derived from human fibrocystic epithelium transfected with Ha-ras) and MCF10AT3B cells (derived from a 94-day third transplant generation of lesion in Beige /Nude mice, classified as grade 2), were kindly provided by Dr. F. R. Miller (Karamanos Cancer Institute, Meyer L. Prentiss Center, Detroit) and grown in RPMI-1640 containing 10% fetal calf serum (FCS). Tissue culture medium was supplemented with penicillin (50 U/ml) and streptomycin (50 μg/ml) (GIBCO-BRL, Gaithersburg, MD) and the cells were maintained at 37°C in a 10% CO_2 humidified incubator. Cells were dissociated with 0.05% trypsin/0.02% EDTA, 0.01M sodium phosphate (pH 7.4) solution (STV) and subcultured at a split ratio of 1:5.

Plasmids and transfection: The DNA and amino acid sequences of ThR are shown in Fig. 1 **[1]**. ThR in the antisense orientation (Fig. 2), consisting of 612 nucleotides (from (-)75 to (+)537 of Fig. 1) was prepared and inserted into the

eukaryotic expression plasmid, pcDNA III (Invitrogene, Carlsbad, CA) at the HindIII and EcoRI sites (Fig. 3). Antisense ThR cDNA was used for transient transfection experiments. Subconfluent (25-40%) MDA-435 breast cancer cells were grown in 60 mm culture dishes and a total of 5-20 μg of DNA and DOTAP - transfection reagent (10 μg DOTAP/μg DNA; 4.5 h incubation, Boehringer Mannheim, Mannheim, Germany) were used for transfection. Cells were assayed 48-72 h following transfection.

RNA Isolation and Northern blot analysis: RNA was prepared using TRI-Reagent (Molecular Research Center, Inc. Cincinnati) according to manufacturer's instructions. The RNA (20 μg of total RNA) was separated by electrophoresis through a 1.1% agarose gel containing 2 M formaldehyde, transferred to a nylon membrane (Hybond N^+; Amersham) and hybridized either to cDNA probes or PCR product radiolabeled by random primer extension with [α-^{32}P]Dct **[6]** for 24 h at 42°C. The membrane was washed twice for 30 min at room temperature with 2x SSC containing 2% SDS and 15 min at 50°C with 0.1x SSC, containing 0.1% SDS. The blots were exposed for 2-4 d at -70°C and the relative amounts of mRNA transcripts were analyzed by laser densitometry using an Ultroscan XL Enhanced Laser Densitometer and normalized relative to internal β-actin controls.

In situ *hybridization of human tumor and placenta biopsy specimens.* RNA probes were transcribed and labeled by T_7 RNA polymerase (for antisense orientation) or T_3 RNA polymerase (for sense control orientation) using DIG-UTP labeling mix (Boehringer Mannheim, Mannheim, Germany). Probes were labeled from plasmid containing 462 base pair fragments of the human ThR (pBhThR-462S) inserted into the EcoRI-HindIII site. Final concentration for hybridization was 1 μg/ml, according to the manufacturer's instructions for non radioactive *in situ* hybridization application. Hybridization was carried out

(overnight, 45°C) on paraffin embedded breast tissue sections (Department of Pathology, Hadassah University Hospital, Jerusalem) or placenta sequential sections. Slides were washed in 0.2xSSPE (3x 1 h) at 50°C and blocked by blocking reagent (Boehringer Mannheim, Mannheim, Germany). Detection was performed using AP-conjugated, anti-DIG antibodies (Fab-fragment, diluted 1:300; Boehringer Mannheim, Mannheim, Germany), overnight at room temperature. AP reaction was detected by NBT/BCIP reagents according to the manufacturer's instructions.

Immunohistochemistry: Tumor cells were cultured overnight at 37°C on eight chamber slides. The cells were fixed with 2% formaldehyde and 2% sucrose/PBS at room temperature for 30 min and permeabilized with 20 mM Hepes, pH 7.4, 300 mM sucrose, 50 mM NaCl, 3 mM $MgCl_2$ and 0.5% Triton X-100, for 4 min at 0°C. After rehydration with PBS, the cells were incubated (10 min, 24°C) with 3% H_2O_2 in PBS containing 10 mM glycine, 10 mg/ml BSA, followed by 30 min blocking with normal goat serum in PBS containing 1% BSA. Affinity purified rabbit-anti-human ThR antibodies were added (dilution 1:50-1:200) for 4 h at 4°C, followed by incubation (1 h, room temperature) with a second antibody goat-anti-rabbit IgG-Biotin conjugated and 1 h incubation with HRP-ExtraAvidin (1:200) (Sigma Immuno Chemicals, St. Louis, MO).

Antibodies: We have raised anti-ThR antibodies directed toward a synthetic peptide (thrombin- receptor activating peptide; TRAP) corresponding to residues Ser42-Lys51 (i.e. S-F-L-L-R-N-P-N-D-K). KLH conjugated peptide was injected to rabbits, and the immune serum was affinity purified. ELISA was performed on plates coated with the TRAP-peptide showing efficient positive identification at 1:25,600 dilution. Maximal response was obtained at 1:3,200

dilution. Monoclonal anti ThR Abs (mouse IgG1 clone IIaR-A) were used for Western blot analysis (Biodesign, ME, USA)

Western blotting analysis: Cells were dissolved in lysis buffer containing 10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100 and protease inhibitors (5 μg/ml aprotinin, 1μM phenylmethylsulfonylfluoride and 10 μg/ml leupeptin) for 30 min at 4°C. After centrifugation at 10,000 g for 20 min at 4°C, the supernatants were transferred and the protein content was measured. Lysates (50 μg) were loaded and resolved on 10% SDS-PAGE followed by transfer to Immobilon-P membrane (Millipore, MA). Membranes were blocked and probed with anti-ThR antibodies (1:4000) in 1% BSA in 10 mM Tris-HCl (pH 7.5), 100 mM NaCl and 0.05% Tween-20). After washes, blots were incubated with the appropriate second antibodies and conjugated to horseradish peroxidase. Immunoreactive bands were detected by the enhanced chemiluminescence (ECL) reagent using luminol and p-cumaric acid (Sigma, St. Louis, Mo).

Placental tissue sections: Sections of placental tissue, 6-15 weeks of gestation, were obtained from elective termination of normal pregnancies by dilatation and curettage.

Matrigel invasion assay: Blind well chemotaxis chambers with 13 mm diameter filters were used for this assay. Polyvinylpyrrolidone-free polycarbonate filters, 8 μm pore size (Costar Scientific Co., Cambridge, MA), were coated with basement membrane Matrigel (25 μg/filter) as previously described [7]. Briefly, the Matrigel was diluted to the desired final concentration with cold, distilled water, applied to the filters, dried under a hood, and reconstituted with serum-free medium. Cells ($2\text{-}3\times10^5$), suspended in DMEM containing 0.1% bovine serum albumin were added to the upper chamber.

Conditioned medium of 3T3 fibroblasts was applied as a chemoattractant and placed in the lower compartment of the Boyden chamber. Assays were carried out at 37°C in 5% CO_2. Over 90% of the cells attached to the filter after a 2h incubation. At the end of the incubation, the cells on the upper surface of the filter were removed by wiping with a cotton swab. The filters were fixed in methanol and stained with hematoxylin and eosin. Cells in various areas of the lower surface were counted and each assay was performed in triplicate. For chemotaxis studies, filters were coated with collagen type IV alone (5μg/filter) to promote cell adhesion. Cells were added to the upper chamber and conditioned medium was applied to the lower compartment.

Examples

Example I: ThR expression in breast carcinoma cell lines.

In a preliminary experiment, a panel of mammary carcinoma cells was surveyed for a possible correlation between the level of ThR expression and established degrees of metastasis (Fig. 4). The cell lines used included one near-normal diploid immortalized breast epithelial cell line (MCF10A) originating from fibrocystic disease, and 6 tumor cell lines exhibiting different doubling times, tumorigenicity and metastases in nude mice. Of these cell lines, MDA-435 (a highly metastatic cell line), and MCF10AT3B (ras transfected fibrocystic epithelium re-established several times from lesions formed in nude mice), were compared to medium metastatic (MDA-231 and MCF10AT, ras transfected fibrocystic cells), or carcinoma cells exhibiting no metastatic potential (ZR-75 and MCF-7 cells). As shown in Fig. 4, high levels of ThR mRNA were found in the highly aggressive cells (lanes A, D) as compared to moderate levels in MDA-231 and ras transfected fibrocystic cells (lanes B& E, respectively), and no expression in the non-metastatic MCF-7 and MCF10AT cells (lanes C&F, respectively). The mRNA levels were quantified by densitometric analysis and the ratio of ThR/β-actin in each lane was calculated. The ThR mRNA level in MDA-435 was 6 fold higher than in MDA-231 cells

(Fig. 4, lanes A vs B) and, as mentioned above, no detectable ThR was observed in MCF-7 cells (Fig. 4, lane C). A similar correlation between ThR level of expression and metastasis was obtained in Ha-ras transfected cells showing a 4 fold higher level in MCF10AT3B (obtained following ras-transfection and xenografting 3 times in mice) than in MCF10AT-ras transfected cells (Fig. 4, lanes D vs E). No detectable level of expression was observed in the fibrocystic, non-malignant, epithelial cells, MCF10A epithelial cells (Fig. 4, lane F).

Affinity purified rabbit-anti-human ThR antibodies were applied to detect the expression and localization of the receptor protein. Massive staining of MDA-231 and MDA-435 cells was observed (Fig. 5B&C, respectively), as opposed to little or no staining of MCF-7 cells (Fig. 5A). In parallel, Western blot analysis showed a distinct protein band of ThR in MDA-435 cells (Fig. 5, lower panel; lane C), somewhat reduced ThR level in MDA-231 (lower panel; lane B) and little or no protein in MCF-7 breast carcinoma cells (lower panel; lane A).

Collectively, these data demonstrate the preferential expression of ThR in metastatic breast carcinoma cell lines, but not in non-metastatic MCF-7 or MCF10A breast carcinoma cells, regardless of whether the mRNA or protein levels were evaluated.

Example 2: ThR expression in human breast tissue specimens.

ThR gene expression and localization *in vivo* was studied in formalin fixed paraffin embedded human breast carcinoma specimens as compared to normal mammary sections obtained from reduction mammoplasty. ThR expression was examined in primary breast tumors representing poor to benign prognosis. *In situ* hybridization analysis using a ThR RNA probe (corresponding to nucleotide nos. 320-570 of the sequence of Fig. 1) was performed with an archival set of paraffin embedded biopsy specimens. A total of 10 normal breast tissue specimens, and 8 specimens of infiltrating ductal carcinoma were analyzed. The invasive carcinoma specimens were selected

from typical infiltrating duct carcinoma of high nuclear grade with numerous atypical mitotic figures and with evidence of vascular invasion and lymph node metastases.

As demonstrated in Fig. 6, hybridization of a ThR antisense RNA probe to invasive duct carcinoma specimens resulted in strong positive staining localized specifically to the carcinoma cells (Fig. 6C). Weaker positive staining was noted in high-grade ductal carcinoma *in situ* (DCIS) of comedo-type (Fig. 6 E&*F*). In contrast, very little or no staining was observed in low-grade, solid type DCIS (Fig. 6G), and no staining was observed in premalignant atypical intraductal hyperplasia (AIDH) (Fig. 6 H&*I*) and in normal breast duct lobular units (Fig. 6 A&*D;* note that the high staining seen in the background is limited to the fibers, and is not seen in the epithelial cells). AIDH was distinguished from low grade DCIS, non-comedo type according to the diagnostic criteria of Dupont, Page and Rogers **[8]**. Expression was also noted in some cases of DCIS, in particular, high grade, comedo-type lesions. The low grade DCIS of solid type showed weak to no expression of ThR, while cases of AIDH, as well as normal breast tissue from reduction mammoplasty specimens did not show any expression of ThR.

Example 3: Antisense ThR inhibits metastatic breast carcinoma cell invasion.

To assess the invasion properties of aggressively metastatic breast carcinoma cells, the Matrigel *in vitro* invasion assay was applied. For this purpose, a reconstituted matrix of basement membrane was utilized to coat porous filters, in order to closely mimic natural barriers in a Boyden chamber. As a chemoattractant source, fibroblast conditioned medium was placed in the lower compartment **[7]**. The Matrigel invasion assay confirmed the expected differential metastatic properties of the carcinoma cell lines. High levels of invasion through Matrigel were obtained with MDA-435 and MDA-231 cells (Fig. 7, D&C). MCF10AT3B-ras transfected fibrocystic cells invaded the

Matrigel to a lower extent (Fig. 7, E), while no movement was detected with the MCF10AT, MCF-7, or ZR-75 non-metastatic cell lines (Fig. 7, F & A ,B, respectively).

To analyze the impact of reduced ThR expression in the highly metastatic cells, MDA-435 breast carcinoma cells were transfected with an antisense ThR cDNA. mammalian expression vector containing ThR cDNA in an antisense orientation under the control of the Cytomegalovirus (CMV) promoter (see Figs. 2 and 3). The vector alone was used as a control. Western blot analysis of ThR protein levels showed a marked reduction in the antisense transfected cells (Fig. 8, lane A) as compared to vector alone (lane B) or untreated MDA-435 cells (lane C). When the antisense transfected cells were tested in the Matrigel invasion assay, the otherwise aggressively invading cells showed a markedly reduced level of invasion, similar to that of the non-metastatic breast carcinoma cell line MCF-7 (Fig. 8, E&F). Transfection with the vector alone had no effect on the invasion properties and the transfected cells migrated effectively through the Matrigel layer (D), similar to the metastatic MDA-435 cells (A).

Similar antisense molecules may be prepared from other members of the PAR family, such as PAR-2 (Fig. 9), PAR-3 (Fig. 10) and PAR-4 (Fig. 11).

Example 4: ThR expression during placenta development.

Human embryo development depends on proper placentation and successful implantation. Trophoblast invasion through the uterine epithelium and deep into the stroma enables the establishment of the proper fetal-maternal interactions. Histological examination of placental biopsies during the first trimester (6-15 weeks), obtained from elective termination of pregnancies, showed a striking pattern of ThR temporal regulation. ThR mRNA levels were not detected up to 6 weeks of gestation (Fig. 12,A), increased markedly between 7-10 weeks (B-*E*), then fell precipitously at 11 weeks and thereafter (F&*G*). The staining was specific to ThR, since hybridization with ThR sense

orientation on placental biopsies taken on weeks 7 and 8, showed no staining (H&I, respectively). The receptor appeared localized to the cytotrophoblasts within the villi, and also, to some extent, in the syncytiotrophoblasts of the invading column.

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CLAIMS:

1. A method for treating metastatic tumor cells of a subject comprising administrating to said subject an antisense molecule, said antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein.

2. A method according to claim 1 wherein said PAR protein is a thrombin receptor.

3. A method according to claim 1 wherein said PAR protein is selected from the group consisting of PAR-2, PAR-3 and PAR-4.

4. A method according to claim 1 wherein said tumor cell is of epithelial tissue origin.

5. A method according to claim 4 wherein said epithelial tissue is selected from the group consisting of breast, esophagus, kidney, prostate, ovary, melanoma and bladder.

6. A method according to claim 1 wherein said antisense molecule has the sequence appearing in Fig. 2.

7. A method for treating metastatic tumor cells of a subject comprising administrating to said subject an antibody molecule, said antibody molecule being capable of binding to a protease activated receptor (PAR) protein.

8. A method according to claim 7 wherein said antibody binds an extracellular epitope of said PAR protein.

9. An antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein.

10. A pharmaceutical composition comprising an active factor and a pharmaceutically acceptable carrier, said active factor being an antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein.

11. A pharmaceutical composition according to claim 10 for the treatment of metastatic tumor cells.

12. A pharmaceutical composition according to claim 11 wherein said PAR protein is a thrombin receptor.

13. A pharmaceutical composition according to claim 11 wherein said PAR protein is selected from the group consisting of PAR-2, PAR-3 and PAR-4.

14. A pharmaceutical composition according to claim 11 wherein said tumor cell is of epithelial tissue origin.

15. A pharmaceutical composition according to claim 14 wherein said epithelial tissue is selected from the group consisting of breast, esophagus, kidney, prostate, ovary, melanoma and bladder.

16. A pharmaceutical composition according to claim 10 wherein said antisense molecule has the sequence appearing in Fig. 2.

17. A method for the treatment of disorders involving the implantation of a placenta in a female subject comprising administrating to said subject an antisense molecule, said antisense molecule comprising a nucleotide sequence which is complementary to an RNA sequence of a protease activated receptor (PAR) protein.

18. A method according to claim 18 wherein said antisense molecule is administered to a trophoblast cell.

19. A pharmaceutical composition according to claim 10 for the treatment of disorders involving the implantation of a placenta in a female subject.

```
   1 gcgcccgcgc gaccgcgcgc cccagtcccg ccccgccccg ctaaccgccc cagacacagc
  61 gctcgccgag ggtcgcttgg accctgatct tacccgtggg caccctgcgc tctgcctgcc
 121 gcgaagaccg gctccccgac ccgcagaagt caggagagag ggtgaagcgg agcagcccga
 181 ggcggggcag cctcccggag cagcgccgcg cagagcccgg gacaatgggg ccgcggcggc
 241 tgctgctggt ggccgcctgc ttcagtctgt gcggcccgct gttgtctgcc cgcacccggg
 301 cccgcaggcc agaatcaaaa gcaacaaatg ccaccttaga tccccggtca tttcttctca
 361 ggaaccccaa tgataaatat gaaccatttt gggaggatga ggagaaaaat gaaagtgggt
 421 taactgaata cagattagtc tccatcaata aaagcagtcc tcttcaaaaa caacttcctg
 481 cattcatctc agaagatgcc tccggatatt tgaccagctc ctggctgaca ctctttgtcc
 541 catctgtgta caccggagtg tttgtagtca gcctcccact aaacatcatg gccatcgttg
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1141 gcaagaagtc ccgggctttg ttcctgtcag ctgctgtttt ctgcatcttc atcatttgct
1201 tcggacccac aaacgtcctc ctgattgcgc attactcatt cctttctcac acttccacca
1261 cagaggctgc ctactttgcc tacctcctct gtgtctgtgt cagcagcata agctcgtgca
1321 tcgaccccct aatttactat tacgcttcct ctgagtgcca gaggtacgtc tacagtatct
```

Fig. 1a

1381 tatgctgcaa agaaagttcc gatcccagca gttataacag cagtgggcag ttgatggcaa
1441 gtaaaatgga tacctgctct agtaacctga ataacagcat atacaaaaag ctgttaactt
1501 aggaaaaggg actgctggga ggttaaaaag aaaagtttat aaaagtgaat aacctgagga
1561 ttctattagt ccccacccaa actttattga ttcacctcct aaaacaacag atgtacgact
1621 tgcatacctg ctttttatgg gagctgtcaa gcatgtattt ttgtcaatta ccagaaagat
1681 aacaggacga gatgacggtg ttattccaag ggaatattgc caatgctaca gtaataaatg
1741 aatgtcactt ctggatatag ctaggtgaca tatacatact tacatgtgtg tatatgtaga
1801 tgtatgcaca cacatatatt atttgcagtg cagtatagaa taggcacttt aaaacactct
1861 ttccccgcac cccagcaatt atgaaaataa tctctgattc cctgatttaa tatgcaaagt
1921 ctaggttggt agagtttagc cctgaacatt tcatggtgtt catcaacagt gagagactcc
1981 atagtttggg cttgtaccac ttttgcaaat aagtgtattt tgaaattgtt tgacggcaag
2041 gtttaagtta ttaagaggta agacttagta ctatctgtgc gtagaagttc tagtgttttc
2101 aattttaaac atatccaagt ttgaattcct aaaattatgg aaacagatga aaagcctctg
2161 ttttgatatg ggtagtattt tttacatttt acacactgta cacataagcc aaaactgagc
2221 ataagtcctc tagtgaatgt aggctggctt tcagagtagg ctattcctga gagctgcatg
2281 tgtccgcccc cgatggagga ctccaggcag cagacacatg ccagggccat gtcagacaca
2341 gattggccag aaaccttcct gctgagcctc acagcagtga gactggggcc actacatttg
2401 ctccatcctc ctgggattgg ctgtgaactg atcatgttta tgagaaactg gcaaagcaga
2461 atgtgatatc ctaggaggta atgaccatga aagacttctc tacccatctt aaaaacaacg
2521 aaagaaggca tggacttctg gatgcccatc cactgggtgt aaacacatct agtagttgtt
2581 ctgaaatgtc agttctgata tggaagcacc cattatgcgc tgtggccact ccaataggtg
2641 ctgagtgtac agagtggaat aagacagaga cctgccctca agagcaaagt agatcatgca

Fig. 1a (Cont.)

```
2701 tagagtgtga tgtatgtgta ataaatatgt ttcacacaaa caaggcctgt cagctaaaga
2761 agtttgaaca tttgggttac tatttcttgt ggttataact taatgaaaac aatgcagtac
2821 aggacatata ttttttaaaa taagtctgat ttaattgggc actatttatt tacaaatgtt
2881 ttgctcaata gattgctcaa atcaggtttt cttttaagaa tcaatcatgt cagtctgctt
2941 agaaataaca gaagaaaata gaattgacat tgaaatctag gaaaattatt ctataatttc
3001 catttactta agacttaatg agactttaaa agcatttttt aacctcctaa gtatcaagta
3061 tagaaaatct tcatggaatt cacaaagtaa tttggaaatt aggttgaaac atatctctta
3121 tcttacgaaa aaatggtagc attttaaaca aaatagaaag ttgcaaggca aatgtttatt
3181 taaaagagca ggccaggcgc ggtggctcac gcctgtaatc ccagcacttt gggaggctga
3241 ggcgggtgga tcacgaggtc aggagatcga gaccatcctg gctaacacgg tgaaacccgt
3301 ctctactaaa aatgcaaaaa aaattagccg ggcgtggtgg caggcacctg tagtcccagc
3361 tactcgggag gctgaggcag gagactggcg tgaacccagg aggcggacct tgtagtgagc
3421 cgagatcgcg ccactgtgct ccagcctggg caacagagca agactccatc tcaaaaaaaa
```

Fig. 1a (Cont.)

MGPRRLLLVAACFSLCGPLLSARTRARRPESKATNATLD
PRSFLLRNPNDKYEPFWEDEEKNESGLTEYRLVSINKSS
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DRFLAVVYPMQSLSWRTLGRASFTCLAIWALAIAGVVPL
VLKEQTIQVPGLNITTCHDVLNETLLEGYYAYYFSAFSAV
FFFVPLIISTVCYVSIIRCLSSSAVANRSKKSRALFLSAAV
FCIFIICFGPTNVLLIAHYSFLSHTSTTEAAYFAYLLCVCVS
SISSCIDPLIYYYASSECQRYVYSILCCKESSDPSSYNSS
GQLMASKMDTCSSNLNNSIYKKLLTZ

Fig. 1b

5'-CGCCGAGGGTCGCTTGGACCCTGATCTTACCCGTGGGCACCCTGCGCTCTGCCTGCC

GCGAAGACCGGCTCCCCGACCCGCAGAAGTCAGGAGAGAGGGTGAAGCGGAGCAGCCCGA

GGCGGGGCAGCCTCCCGGAGCAGCGCCGCGCAGAGCCCGGGACAATGGGGCCGCGGCGGC

TGCTGCTGGTGGCCGCCTGCTTCAGTCTGTGCGGCCCGCTGTTGTCTGCCCGCACCCGGG

CCCGCAGGCCAGAATCAAAAGCAACAAATGCCACCTTAGATCCCCGGTCATTTCTTCTCA

GGAACCCCAATGATAAATATGAACCATTTTGGGAGGATGAGGAGAAAAATGAAAGTGGGT

TAACTGAATACAGATTAGTCTCCATCAATAAAAGCAGTCCTCTTCAAAAACAACTTCCTG

CATTCATCTCAGAAGATGCCTCCGGATATTTGACCAGCTCCTGGCTGACACTCTTTGTCC

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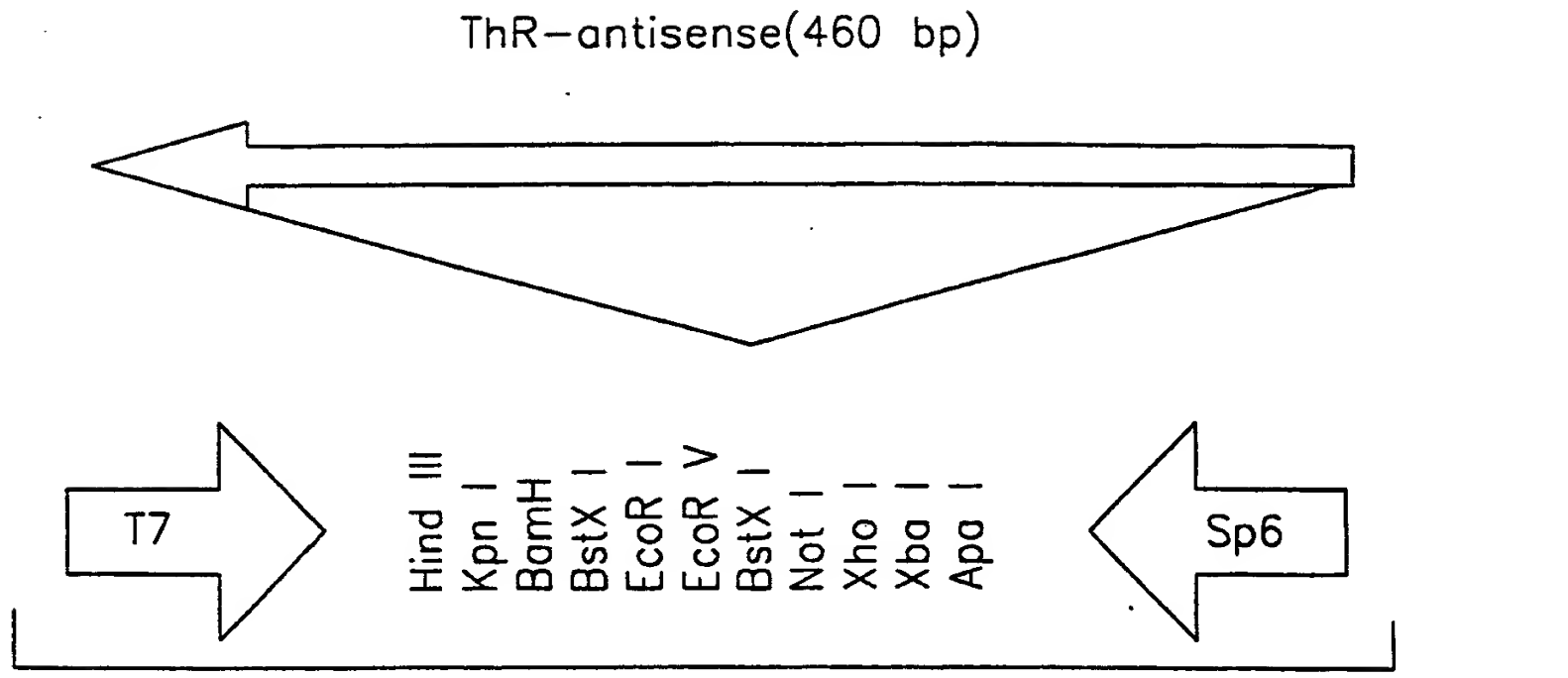
TGTTCATCCTG-3'

Fig. 2


ThR–antisense(460 bp)
T7
Hind III
Kpn I
BamH
BstX I
EcoR I
EcoR V
BstX I
Not I
Xho I
Xba I
Apa I
Sp6

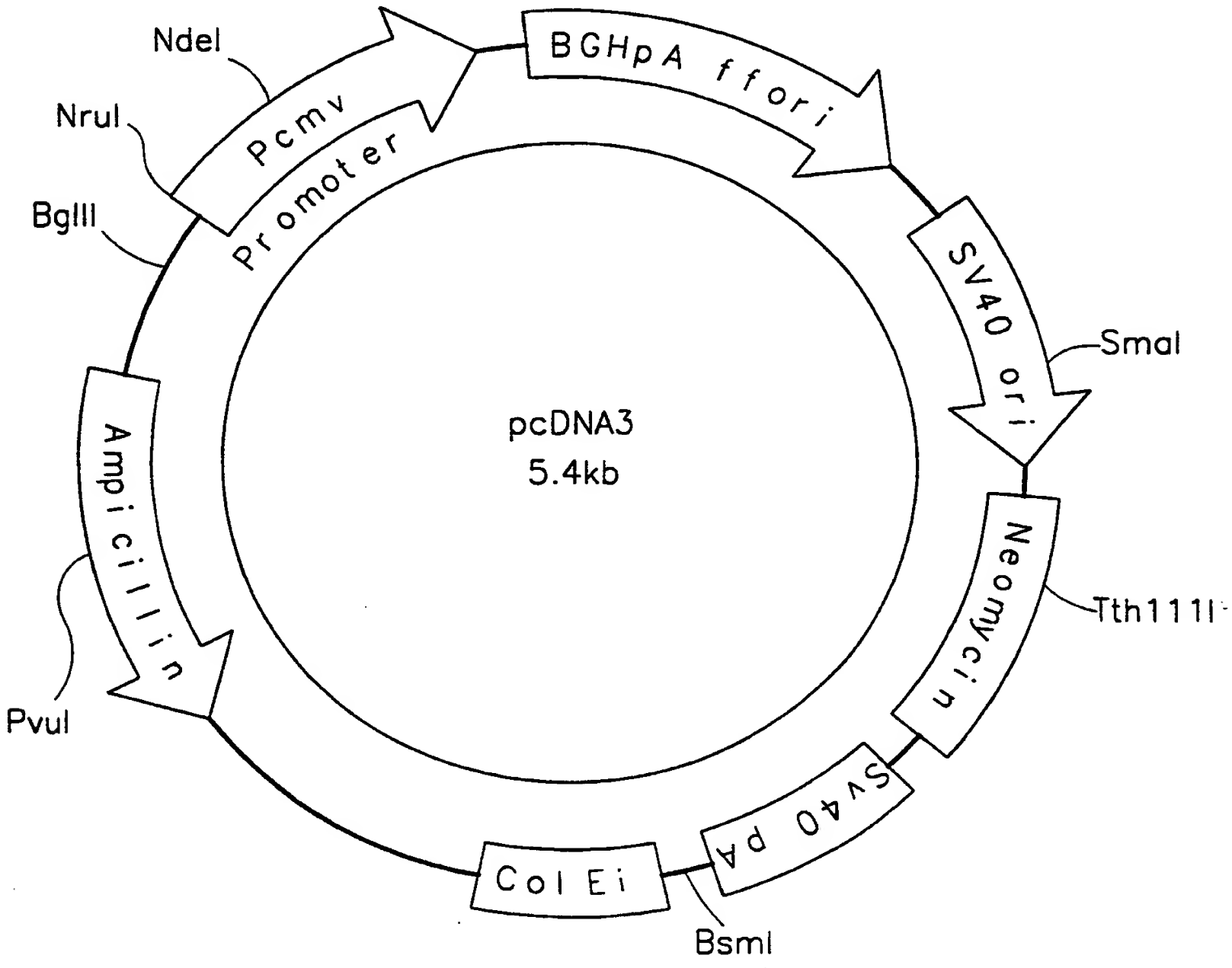

Ndel
Nrul
BglII
Pvul
Pcmv Promoter
BGHpA
f1 ori
SV40 ori
SmaI
Neomycin
Tth111I
SV40 pA
Col Ei
BsmI
Ampicillin
pcDNA3
5.4kb

FIG.3

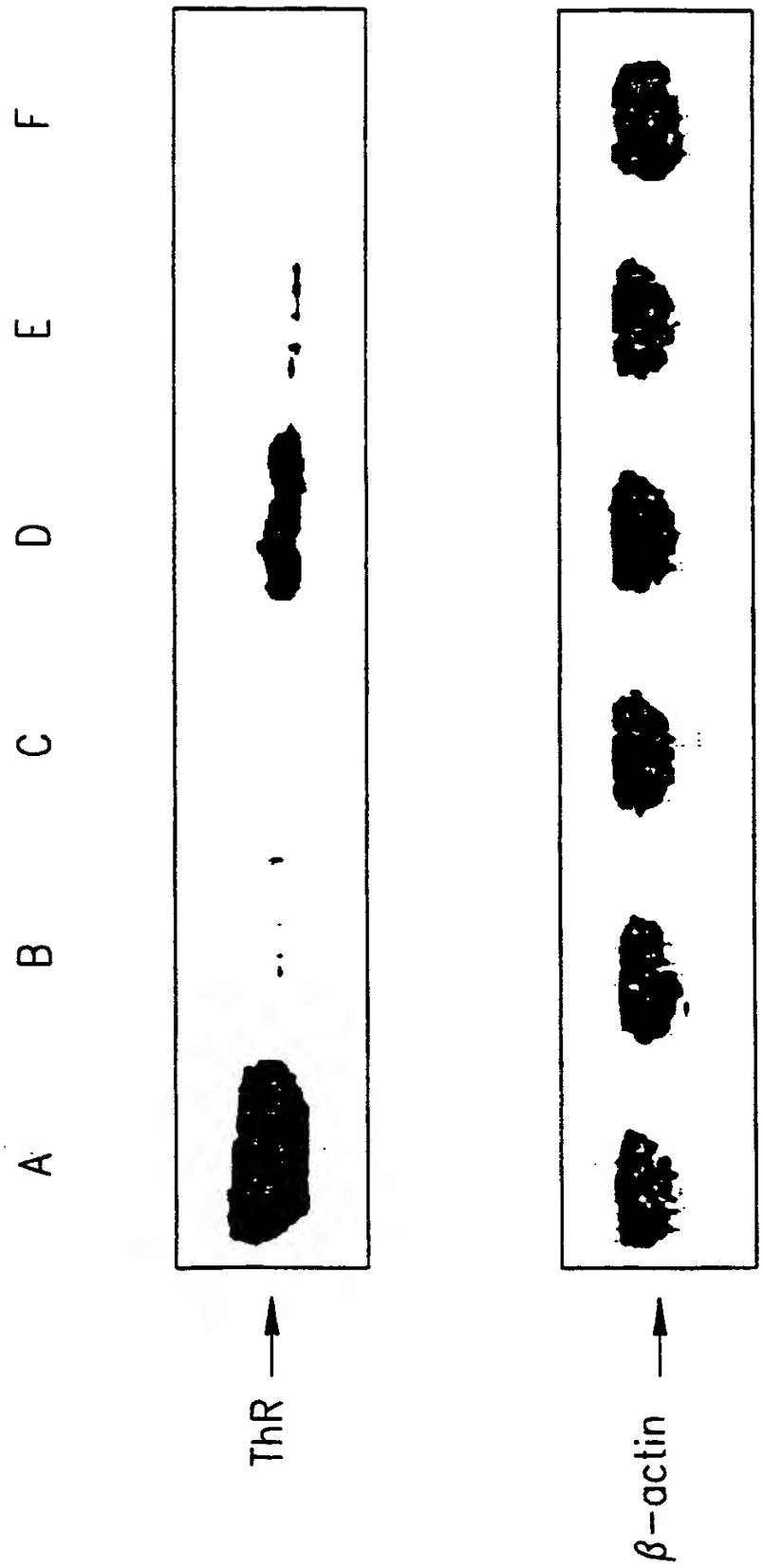
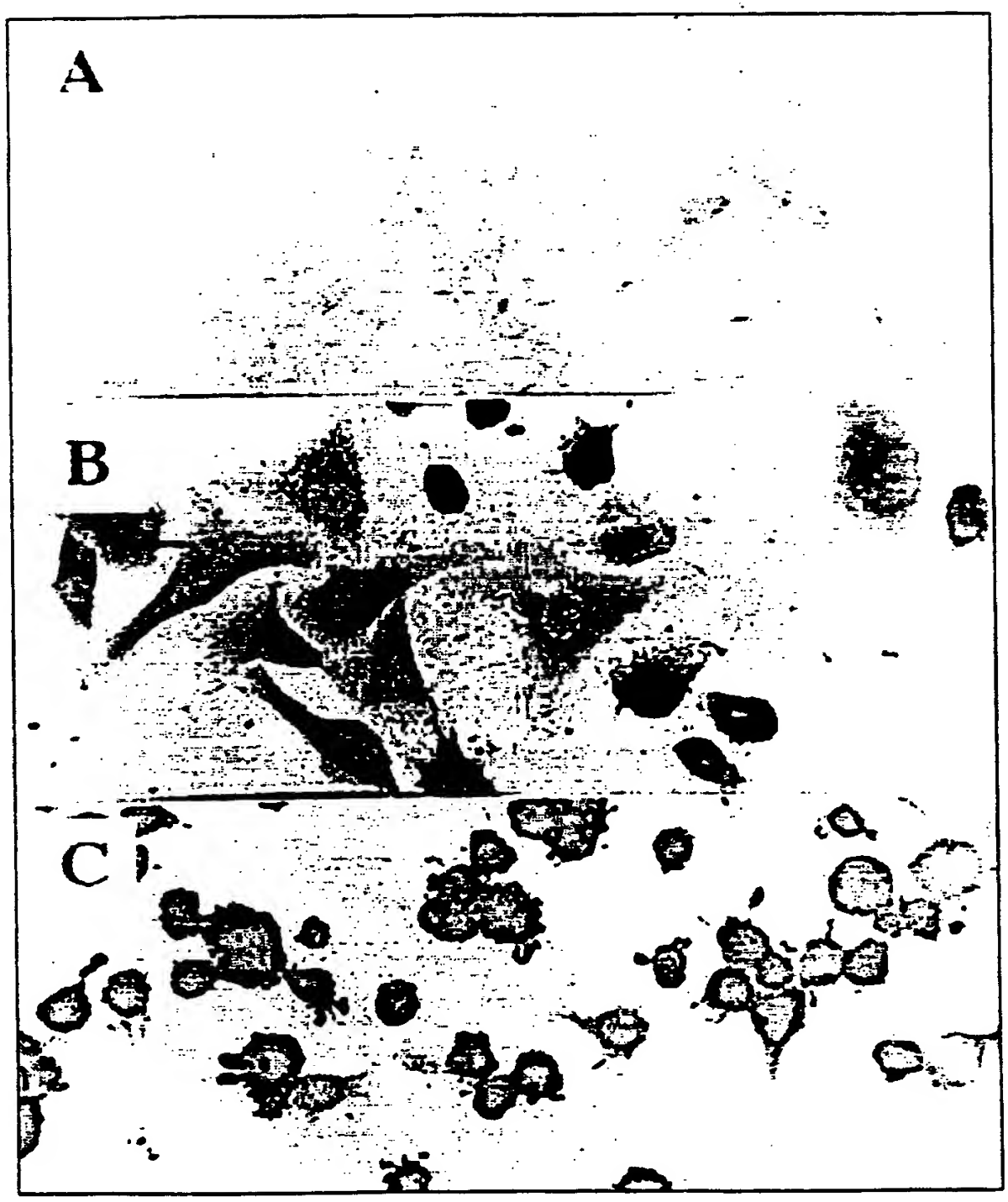


FIG.4



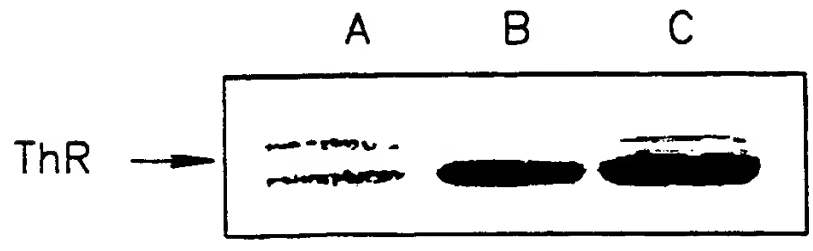


FIG.5

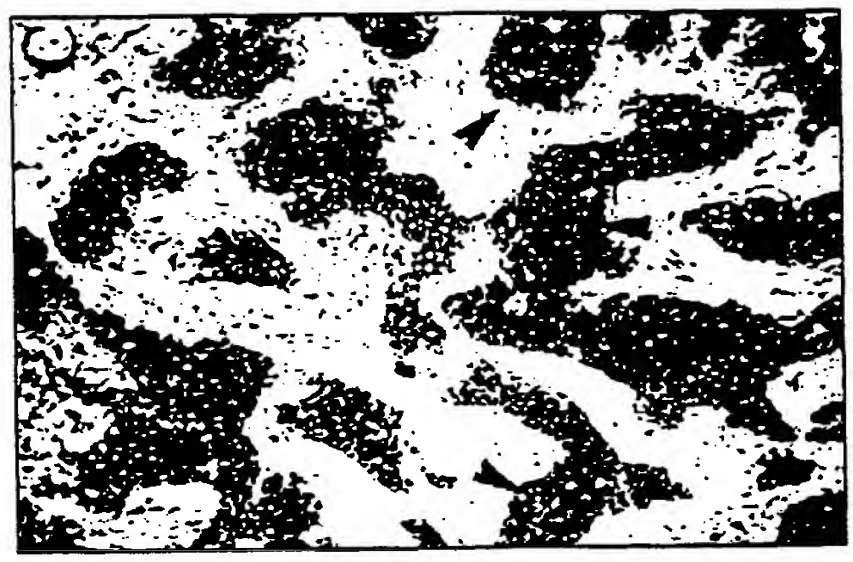


FIG.6C

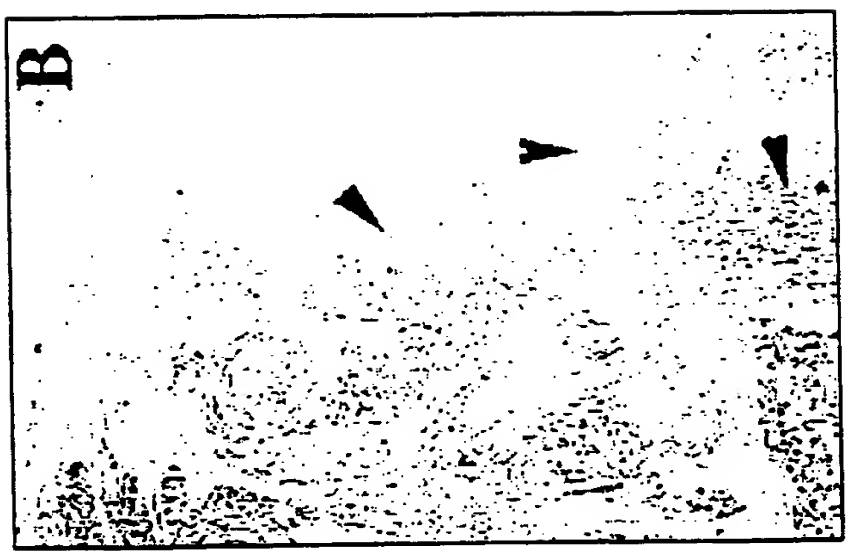


FIG.6B

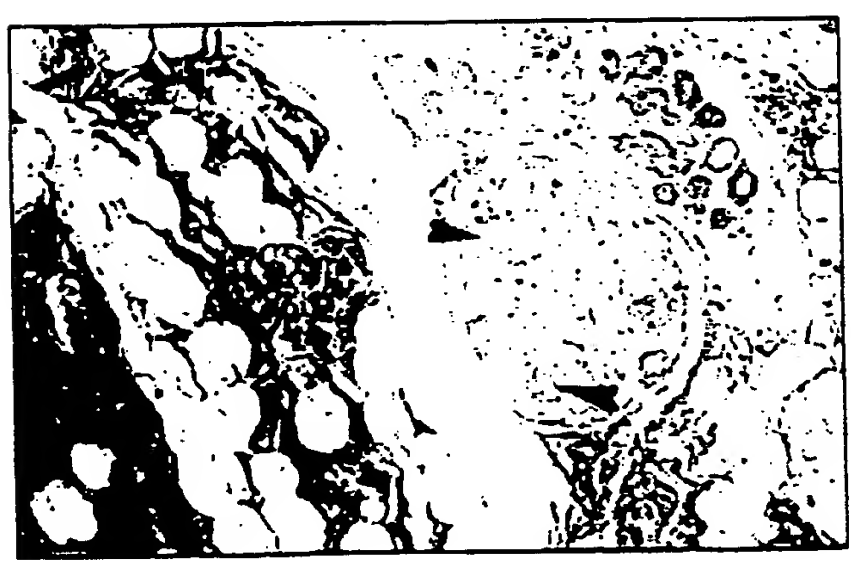


FIG.6A

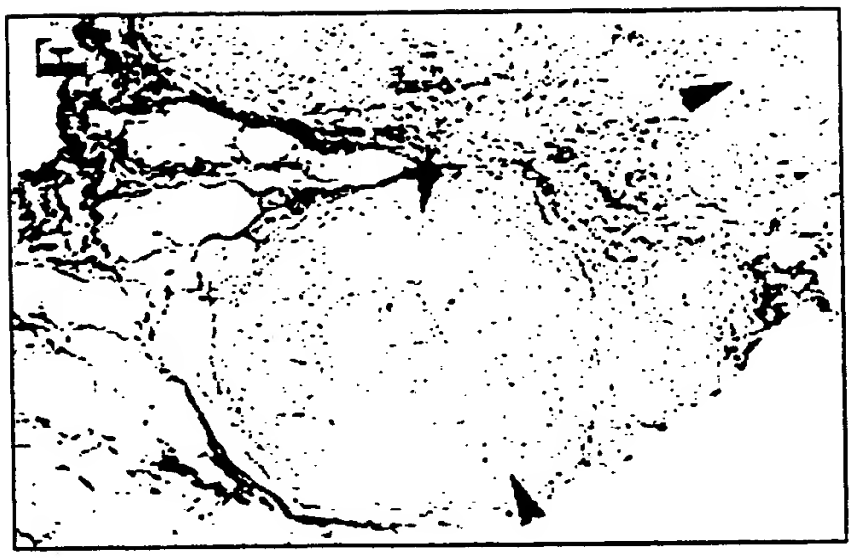


FIG.6F

FIG.6E

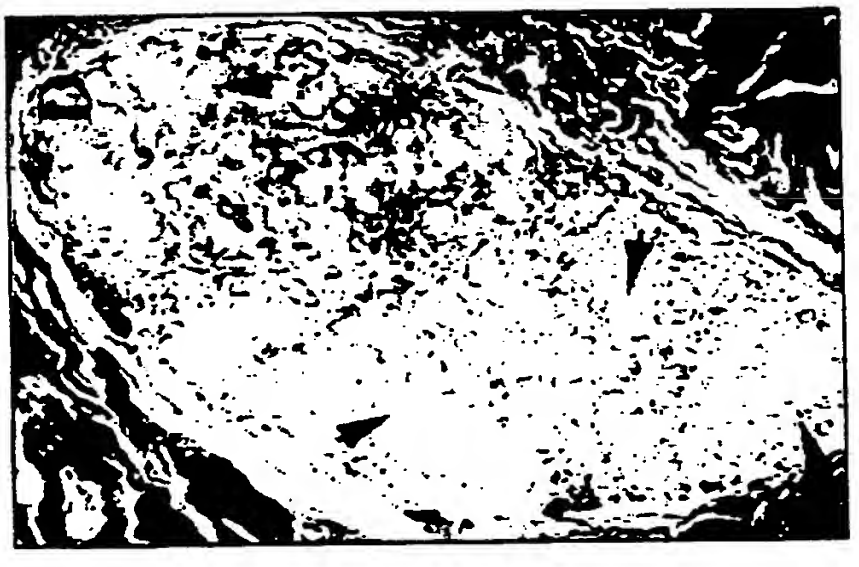


FIG.6D

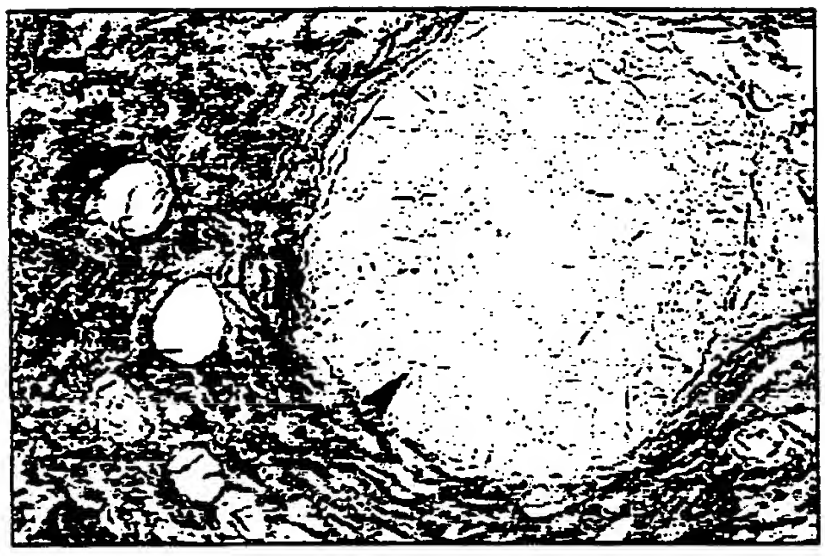


FIG.6I

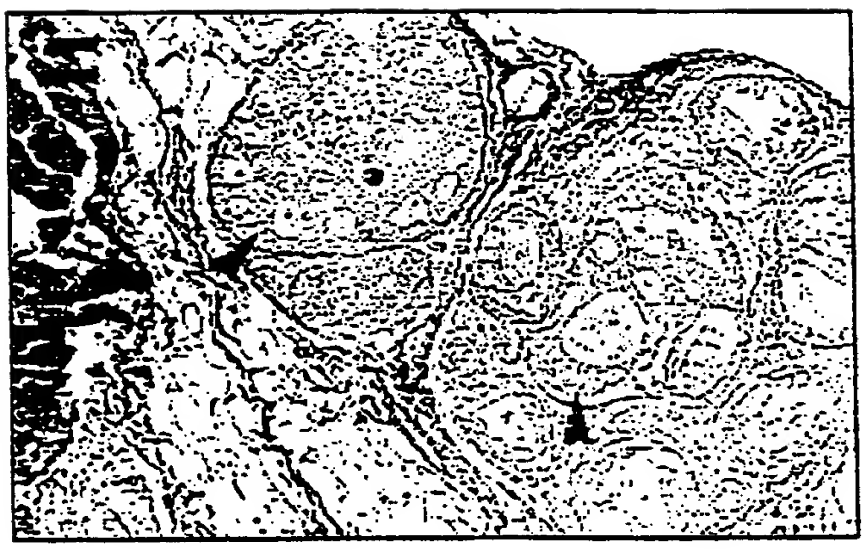


FIG.6H

FIG.6G

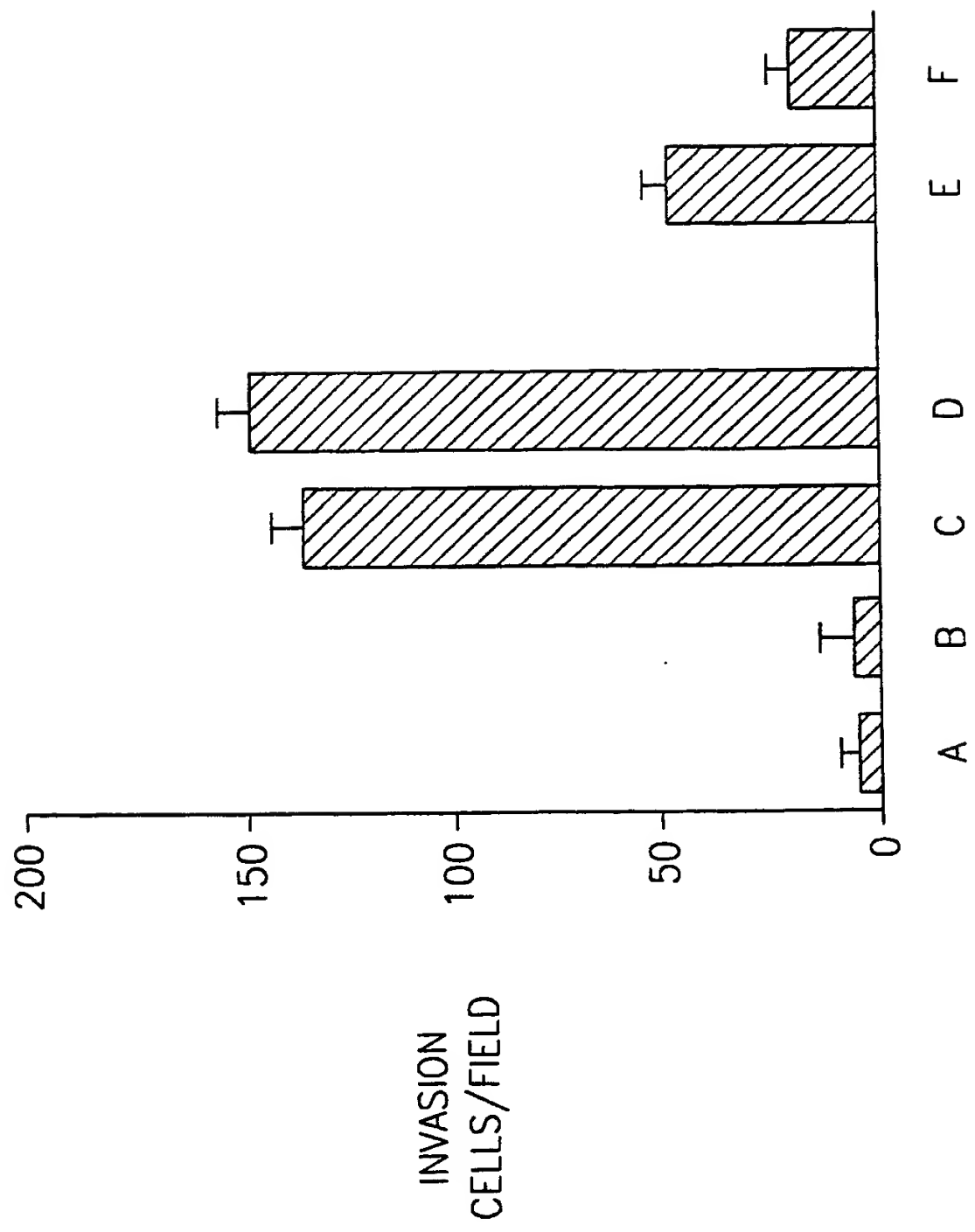
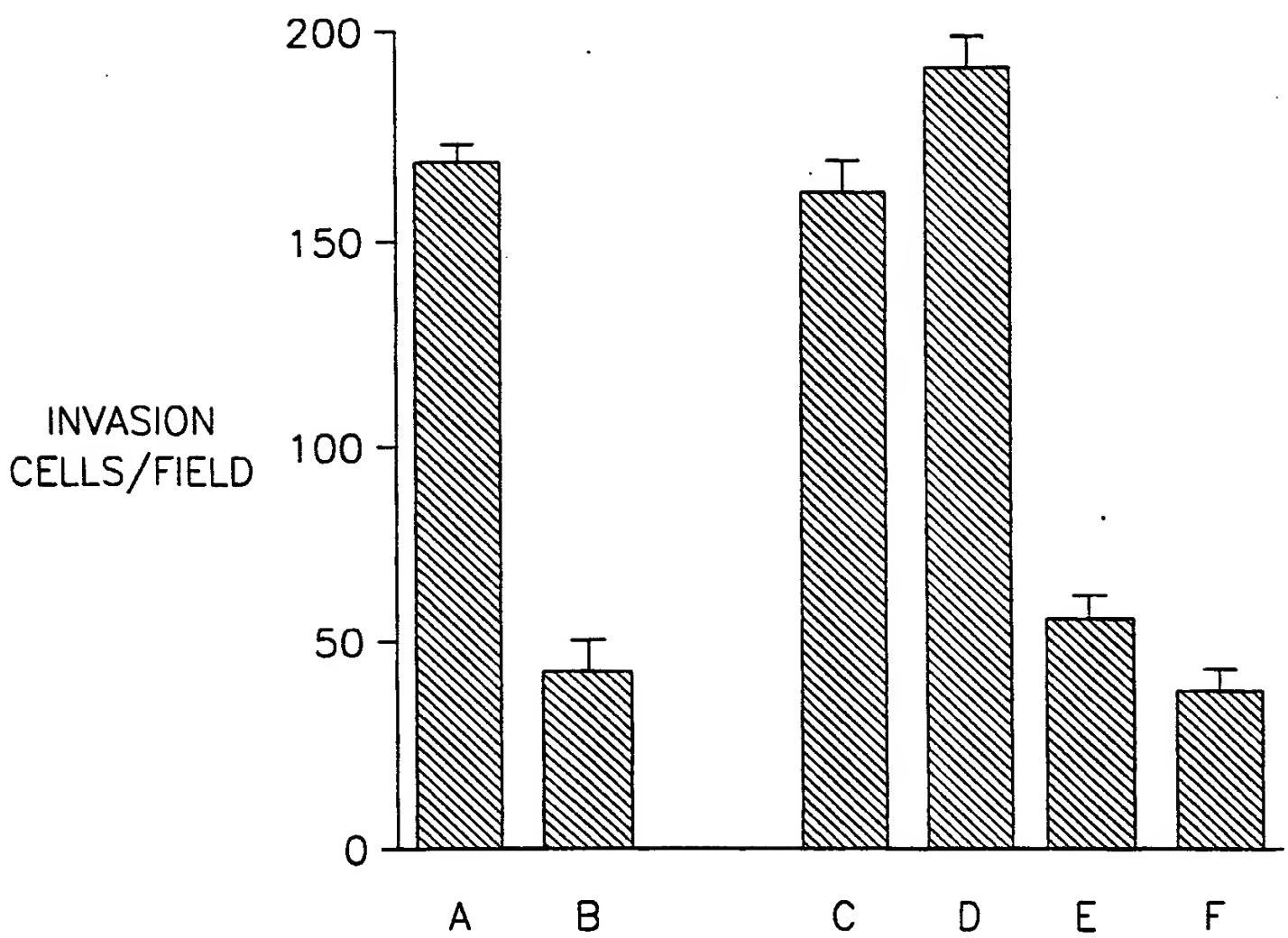


FIG.7



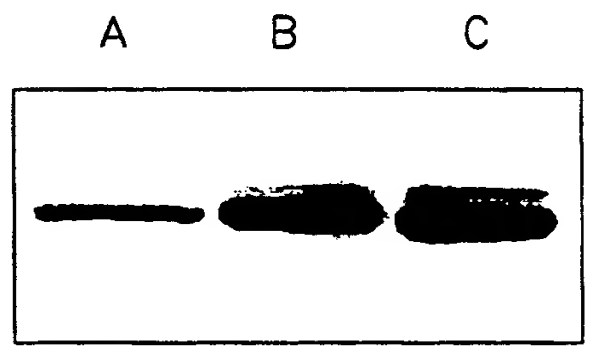


FIG.8

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 421 tacatggcaa caactggatt tatggggaag ctctttgtaa tgtgcttatt ggctttttct
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1261 atgttatgag gacgtgtctg ttatttcct
```

Fig. 9

```
   1 cggcacgagc aaggacgagt ccctgcccac acagtccagg ctggcagagt tctcagcttt
  61 ccacttgctg ctcatacatg gagctgaggg gaatctaccc tgtgactttg tatacttaac
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1201 gctgcctaga tccattcctt tactttgtca tgtcgaaagt tgtagatcag cttaatcctt
1261 agtcggcaat ggcaagacca ctttagagac caaggagaga tatctgggaa gacatacatg
1321 cttggctgac ttatgcatgg caccaacagc tcaattttta attttaattt aattttatct
1381 ttttgagaca gaacctcact gtgtagtcct ggctggcctg gctgattctc tatttagacc
1441 aggttagcct tgaactcaca gagatctgcc tgcttctgcc tcccaagtgc tgggttcaac
1501 caggtctggc aagcgctcca tttgtcagct cctctgcaac agtgctttat acttccaatg
1561 tgaagtcatg aggattaaaa gaatactttg tatttagaga gtgtcttaat tgtaagcaac
1621 attagcagta ttccgatggc aatggacgca tttctatttg ccttcctgat tctccaagct
1681 atacatgtcc ccttcctctc tgtgagcggt gtcggcagac tgggaggttg tcactacccg
1741 tggttctccc tcctcctcaa gcaggtatcc tgtctttgtt gtgtctctca ctggggtccc
1801 gtgttcatgc cacctctgtc caaagatggc agcctcactg ccagccgagc ctcatttcca
1861 cagattgggt taaccttccc tgaacagttt ctgaatacat catgcaatac ttcacttgac
1921 cctgcagtgc tcctgaaatg acaagccctt gcatggcttt cctggtggag ctgtgcctgc
1981 gattccacac accttcacac cgactgtctt ccctcccatc cctgtcgctc cactgaaacc
2041 ccttctagca tctttgcttt agcctgtcca gcttttcaag tcgttaaagg cccattcttc
2101 ccttattctc aaactaaaaa catcattttc tttatctgga tttgtaaagc actttcttat
2161 ctttaaggca cttctagaca aacaagcctg ctaagctcat ttgcatatag atattacttc
2221 cataaggaat taagttctct gtgtggtcag ctctgtatcc ctgcaagttt catacctgta
2281 gattcaacca accacagacc caaaacactt gaaaaacctt gtatgagctg aaaacgaaga
2341 gttctatttt tcattactcc ntaaataata ntgataataa ataataataa ataaaaaaaa
2401 aaaaaaaaa
```

Fig 10

1 ctcccacggg ctggctggca agcggccctg gtgggtctgc gggggcaggg gcagccttcc
61 tggtttatct ccaccggcgc gatctgctcg tccgcctcgg ctccagaagc tggggctcag
121 ggtccggcga ggcaggaagc ctgaggccac agcccagagc agcctgagtg cagtcatgtg
181 ggggcgactg ctcctgtggc ccctggtgct ggggttcagc ctgtctggcg gcacccagac
241 ccccagcgtc tacgacgaga gcgggagcac cggaggtggt gatgacagca cgccctcaat
301 cctgcctgcc ccccgcggct acccaggcca agtctgtgcc aatgacagtg acaccctgga
361 gctcccggac agctcacggg cactgcttct gggctgggtg cccaccaggc tggtgcccgc
421 cctctatggg ctggtcctgg tggtggggct gccggccaat gggctggcgc tgtgggtgct
481 ggccacgcag gcacctcggc tgccctccac catgctgctg atgaacctcg cgactgctga
541 cctcctgctg gccctggcgc tgcccccgcg gatcgcctac cacctgcgtg gccagcgctg
601 gcccttcggg gaggccgcct gccgcctggc cacggccgca ctctatggtc acatgtatgg
661 ctcagtgctg ctgctggccg ccgtcagcct ggatcgctac ctggccctgg tgcacccgct
721 gcgggcccgc gccctgcgtg gccggcgcct ggcccttgga ctctgcatgg ctgcttggct
781 catggcggcc gccctggcac tgcccctgac actgcagcgg cagaccttcc ggctggcgcg
841 ctccgatcgc gtgctctgcc atgacgcgct gcccctggac gcacaggcct cccactggca
901 accggccttc acctgcctgg cgctgttggg ctgtttcctg cccctgctgg ccatgctgct
961 gtgctacggg gccaccctgc acacgctggc ggccagcggc cggcgctacg gccacgcgct
1021 gaggctgacc gcagtggtgc tggcctccgc cgtggccttc ttcgtgccca gcaacctgct
1081 gctgctgctg cattactcgg acccgagccc cagcgcctgg ggcaacctct atggtgccta
1141 cgtgcccagc ctggcgctga gcaccctcaa cagctgcgtg gatcccttca tctactacta

Fig. 11a

1201 cgtgtcggcc gagttcaggg acaaggtgcg ggcagggctc ttccaacggt cgccggggga
1261 caccgtggcc tccaaggcct ctgcggaagg gggcagccgg ggcatgggca cccactcctc
1321 tttgctccag tgacacaaag tggggaaggc tgtactgggt cgaacagggt cccttccccc
1381 acttcacgtc cttcctggga cctcagaatg tgaccttatt tggaaatagg gttgttacaa
1441 ctgtcactag cggaggtcac tttggagaag ggtgggcctt acatccagtg tgggtggtgt
1501 cctcataaga taaggagagg ccaggcctgg tggctcacgc ctgtaatccc agcactttaa
1561 gaggccaagg cggatggatc acttgagccc aggagttcaa caccagcctg agcaacatgg
1621 taaaacccca tctctaccaa aaatacaaaa attagctggg cttggtggct ggcgcctgta
1681 atcccagcta ctcaggagac tgaggcagaa ggatcgcttg aacctgggag gcagaggttg
1741 cagtgagccg agattgcgcc actggactcc agcctgcgtg acagagagcc tgtctctaaa
1801 ttaattaatt aattaattta attcaatttt aaaaagacga aaagtgacgg ccaggtgcag
1861 tggctcacgc ctataatctc agcactctgg gaggccaaga tggaggattg cttgaagcca
1921 ggagtttggg accagcctgg gcaacatagg gggatcccat ctctacacac aaaaaaattt
1981 tttaatgaac caggcattgt ggcatgcgcc tatagtccca gccactcaag aggcacaggc
2041 gggaggatca cttgagcctg ggaggttgtg gttgcagtga gctatgattg taccactgca
2101 ctccagcctg ggcaacagag caagaccttg tctcaaaaat aaacaaacta aaattaaaaa
2161 aagaagacga gagatagtgg gtgtggtggc tcacacctgc aatcccagca ctttggaagg
2221 ccgaggtggg cagatcatct gaggccagga gttcaagacc agcctggcta acatggtgaa

Fig. 11a (Cont.)

2281 atcctatctc taccaaaaat acaaaaatta gccaggcgtg gtggtgggca cctgtactgg
2341 ggaggtgccc acccagctac tggggaggct gagtcaggag aatcgcttga acctgggagg
2401 cggaggttgc ggtcagctga gatggtgcca ctgcactcca gcctgggcga aagagcgact
2461 ctgtctccaa aaaaaagaga agaggagagg acacagagac acacagagaa gaaagccatg
2521 tggcggcaga ggcagagatg ggagtgatgc ggacggacac aaactaaggg atgccacgat
2581 gccaagcaca gccaacagcc accagcagcc aggagacagg cctgggacgg gctctccctc
2641 acagcctcca gagggaacca gccctgccac caccttgacc ctggacttct ggcctgcaga
2701 actgtgagac aataaactct cattgtttta agctgcctgg catgtggcac tttgtcaggg
2761 cagcccagga atctgaaaca ggatcaaact ctgcttcctg ggccctgcca gcatctctgg
2821 ctcggctttc tgggctggat gcagcccacg acgcactggt gtctgagatg gggctggagc
2881 tggggctggg gctgcattcc ctggagactc actgcaagtt cctgcccagg aggctgaggg
2941 caccccatcc tcagtgccca atgctgtggc cccaccaggc ccagagcctg gttggccatt
3001 ctcatgccca ccagcttctg gctttgggat gtctcttgag caaccagaat agcacccccа
3061 actctgctcc ccaaaaccca tcactagcac ggctcagcct cctgctatcc cctgactgct
3121 ggggaccctc gccttccctc ctctcacctg caggctgatc cttcttttca ctttctgtca
3181 atgtcaccag ggataaggtg ggacaatggg gggtgggggt ggacagtgtg tgctgggggg
3241 ttcgggtgct gcagacctgg aactcccttc tgccaggatg ttggcagccg gttgtaagcc
3301 ttgcacggga cagaccacac ccaccgcaac ctcatcccct cagcactaac cacatccact

Fig. 11a (Cont.)

3361 ctcaaccccg tcccttcgc actgaccaca cccaccccgt tcggccccgc cccccgcact
3421 gaacactccc gccctcaacc ccgcaccctc cgcactcacc tccccctcgc cgctcgaccc
3481 cgccctcacc acactgacca ccctcaaccc attgcgccca gtccccacca cagtgaccac
3541 accctcactg gctcggccct gcccccagta tactgaccat tccccagcca cttcccttcc
3601 gcacttacca ctcccccagc cacgcccctc cccgctgacc gctcctccag ccccgcctcc
3661 cccgtacagg cagagcgccc gcccacctct atgctgcgtt ctcctgactt tacgttggcc
3721 cctcctctgc caagccccca ggggagccct ccctggcgtc cgagggtggg agtcggggtg
3781 tggcaggccg cggtgggggg cggcagtggc tccgcgcact cacccgggcc ccgggcaggg
3841 gcgcgctcca cttcgttgca cgcgggtccg gcgcacagtt cccgggcgag tgggctgtgc
3901 gtgctgacgt tgtagaagcg agtggcctcg aaggctacgg gacgagggtg gcgggtgacc
3961 aagtgcaggc gcgacgggtc agggaccggg ccgggccggg ggtgcgggcg cgcgggccta
4021 ccgggttcgt agtagtcgta cacggagact ggcagcgccg acgtcctgcc caccacgcac
4081 tcccggagag cacggaaccg cacgcacgtc aggcaccggc tggggatctg tggggcagcg
4141 gcgggcgcag gctcgacccg ggccaggagg cccggggcgc tgagctcagg cccagaactg
4201 gctgatttca gggataccca ggacgcgtga aacacagaag aaacgtgatc ccatttttctt
4261 ttttctttt actttcttt ttttttttt ttcctgagac agagtctcgc gctgttgccc
4321 aggctggagt gcagtggcgt gatctcggct cactgcaagc tcggcctcct gggttcaaat
4381 gattctcctg cctcagcctc ccaagtagct gggataacag gcgcccacca ccgcaccctg

Fig. 11a (Cont.)

4441 ctaatttttt gtatttttga tcaagacgga gtttcaccat gttggccagg ctggtctcca
4501 actcctgccc tcaagtgatc cgcctcggtc ccatttttta ttctttgggt ccttccatcc
4561 cactgggaaa acgtctcagg tggcctctga aacaccactc ctttttgtgt gtgtgcacgc
4621 atggctgagc atgtgtgggt gggagtcagc acattcacga tactgtgcaa tcatcacctc
4681 tgtctagtta caggacggtt tctttctccc ccaaagaaac cccatcgcca tcagcactca
4741 ctccccactc ccccagcccc tggcaaccac aaatctttcc aactctacgg atttgcctgt
4801 tctgggcatt tcatgtcaat ggaatcatgt actctgtgaa aaaaaaaaaa aaaaaaaaaa
4861 aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaa

Fig. 11a (Cont.)

MWGRLLLWPLVLGFSLSGGTQTPSVYDESGSTGGGDDSTPSILP

APRGYPGQVCANDSDTLELPDSSRALLLGWVPTRLVPALYGLVLVVGLPANGLALWVL

ATQAPRLPSTMLLMNLATADLLLALALPPRIAYHLRGQRWPFGEAACRLATAALYGHM

YGSVLLLAAVSLDRYLALVHPLRARALRGRRLALGLCMAAWLMAAALALPLTLQRQTF

RLARSDRVLCHDALPLDAQASHWQPAFTCLALLGCFLPLLAMLLCYGATLHTLAASGR

RYGHALRLTAVVLASAVAFFVPSNLLLLLHYSDPSPSAWGNLYGAYVPSLALSTLNSC

VDPFIYYYVSAEFRDKVRAGLFQRSPGDTVASKASAEGGSRGMGTHSSLLQ

Fig. 11b

FIG.12C

FIG.12B

FIG.12A

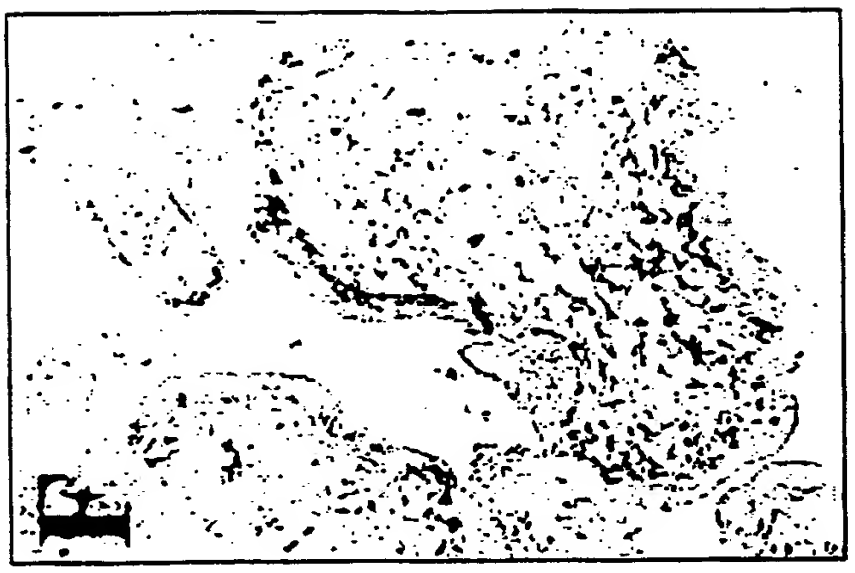

FIG.12F

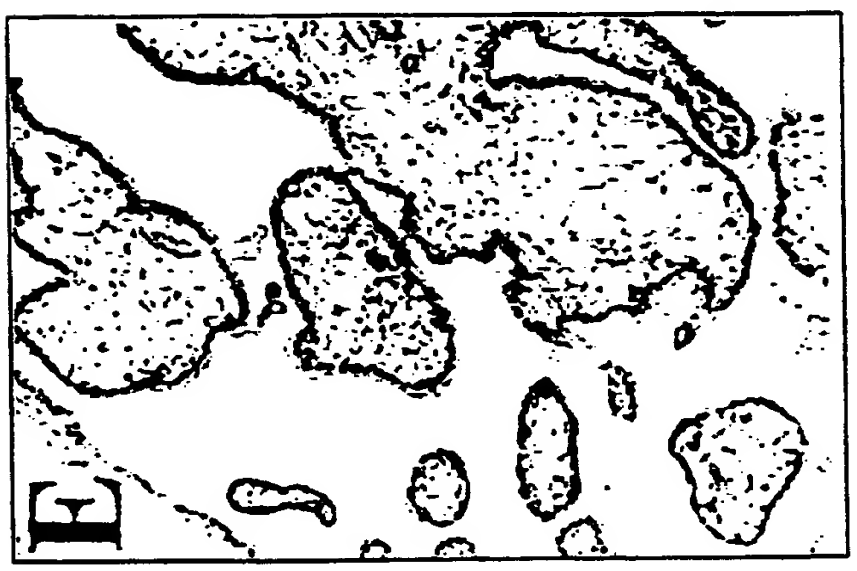

FIG.12E

FIG.12D

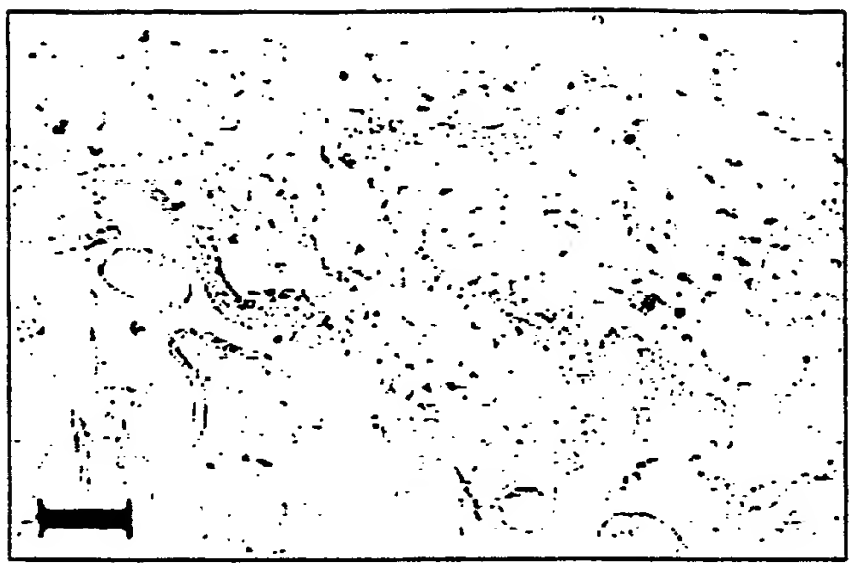

FIG.12I

FIG.12H

FIG.12G

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL99/00079

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/12; A61K 37/02
US CL :536/23.5; 435/69.1, 325, 320.1; 514/13

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 536/23.5; 435/69.1, 325, 320.1; 514/13

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,352,664 A (CARNEY et al) 04 October 1994, see entire document.	1-19
A,E	US 5,892,014 A (COUGHLIN et al) 06 April 1999, see entire document.	1-19

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
01 JUNE 1999	15 JUN 1999

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